B16-Blue™ IFN-α/β Cells
Murine Type I IFNs Sensor Cells
Catalog # bb-ifnt1
For in vitro use only
Version # 16I13-MM

PRODUCT INFORMATION
Contents
• 1 vial of B16-Blue™ IFN-α/β cells (3-7 x 10⁶ cells) in freezing medium
IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.
• 100 µl Zeocin™ (100 mg/ml). Store Zeocin™ at 4 °C or at -20°C.*
• 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*
*The expiry date is specified on the product label.
• 1 pouch of QUANTI-Blue™ (SEAP detection medium). Store QUANTI-Blue™ pouch at 4°C for 12 months. Reconstituted medium is stable for 2 weeks at 4°C. Keep reconstituted QUANTI-Blue™ away from light.

Handling Cells Upon Arrival
Cells must be thawed immediately upon receipt and grown according to handling procedures to ensure the best cell viability and assay performance. If you are unable to thaw the cells immediately, frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

Product Warranty
InvivoGen warrants that cells shall be viable upon shipment from InvivoGen for a period of thirty days, provided they have been properly stored and handled during this period.

Cell Line Stability
Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. B16-Blue™ IFN-α/β cells should not be passaged more than 20 times to remain fully efficient. B16-Blue™ IFN-α/β cells should be maintained in Growth Medium supplemented with Zeocin™ (100 µg/ml). Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for SEAP.

Quality Control
Reporter activity is validated by stimulating the cells with murine IFN-α (mlIFN-α), mlIFN-β and type I IFN activators. These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS
These cells are distributed for research purposes only.
This product is covered by a Limited Use License. By use of this product, the buyer agrees the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com

INTRODUCTION
Interferon-alpha (IFN-α) and interferon beta (IFN-β), play an important role in viral infections. They bind to an IFN receptor complex consisting of two alpha chains (IFNAR1 and IFNAR2) and recruit JAK1 and Tyk2. These kinases phosphorylate STAT1 and STAT2 leading to the formation of the ISGF3 complex. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression. IFN-α and IFN-β are produced in response to viral pathogen associated molecular patterns (PAMPs), such as viral RNA and DNA. Stimulation of B16-Blue™ IFN-α/β cells with dsRNA delivered intracellularly, triggers the secretion of IFN-α and IFN-β.

CELL LINE DESCRIPTION
B16-Blue™ IFN-α/β cells allow the detection of bioactive murine type I IFNs by monitoring the activation of the JAK/STAT/ISGF3 pathway. They derive from the murine B16 melanoma cell line of C57BL/6 origin after stable transfection with a SEAP reporter gene under the control of the IFN-α/β-inducible ISG54 promoter enhanced by a multimeric ISRE. B16-Blue™ IFN-α/β cells do not respond to IFN-γ, due to the inactivation of IFN-γ receptor. B16-Blue™ IFN-α/β cells respond specifically to mlIFN-α/β and do not respond to human IFN-α/β. Stimulation of B16-Blue™ IFN-α/β cells with murine IFN-α or IFN-β, or type I IFN inducers, such as poly(I:C), poly(dA:dT) or 5’ppp-dsRNA delivered intracellularly, triggers the production of SEAP by the activation of the IRF-inducible promoter. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP and by reading the OD at 655 nm.
B16-Blue™ IFN-α/β cells are resistant to Zeocin™.

Detection range: 10⁵ - 10⁶ IU/ml for mlIFN-α/β
SAFETY CONSIDERATIONS

Biosafety Level 1

Required Cell Culture Medium
- Growth Medium: RPMI, 10% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine
- Freezing Medium: RPMI, 20% fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine, 10% (v/v) DMSO
- Test Medium: RPMI, 10% (v/v) heat-inactivated fetal bovine serum (30 min at 56 °C), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

Note: Heat-inactivated FBS is also commercially available.

Required Selective Antibiotic(s)
- Zeocin™

HANDLING PROCEDURES

Initial Culture Procedure
The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

- Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
- Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

Note: All of the operations from this point should be carried out under strict aseptic conditions.
- Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. Do not add selective antibiotics until the cells have been passaged twice.
- Centrifuge vial at 1000-1200 RPM (RCF = 200-300 g) for 5 minutes.
- Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
- Transfer the vial contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
- Place the culture at 37 °C in a CO₂ incubator for 20-24 h.

Frozen Stock Preparation
1. Resuspend cells at a density of 3-5 x 10⁶ cells/ml in freezing medium prepared extemporaneously with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
2. Aliquot 1 ml cells into cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell maintenance
- Maintain and subculture the cells in growth medium supplemented with 100 µg/ml of Zeocin™.
- Renew growth medium twice a week.
- Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Reported Assay

Day 1:
- Add 20 µl of each sample per well of a flat-bottom 96-well plate.
- Add 20 µl of positive control, such as murine IFNα/β 10⁴ U/ml or Type I IFN inducer, in one well.
- Add 20 µl of negative control (test medium or sterile PBS) in one well.
- Prepare a cell suspension of B16-Blue™ IFN-α/β cells at ~420,000 cells per ml in test medium (containing 10% v/v heat-inactivated FBS).

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive.

- Add 180 µl of cell suspension (~75,000 cells) per well.
- Incubate the plate at 37 °C in a CO₂ incubator for 20-24 h.

Day 2:
- Prepare QUANTI-Blue™ following the instructions on the enclosed product data sheet.
- Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
- Incubate the plate at 37 °C incubator for 1-5 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
QUANTI-Blue™
Medium for detection and quantification of alkaline phosphatase
Catalog # rep-qb1, rep-qb2
For research use only
Version # 16C18-MM

PRODUCT INFORMATION
Contents:
QUANTI-Blue™ is provided as packs of individually sealed pouches.
• rep-qb1: 5 pouches of QUANTI-Blue™
• rep-qb2: 10 pouches of QUANTI-Blue™
Each pouch contains everything needed to prepare 100 ml of medium for the detection and quantification of any alkaline phosphatase.

Storage and Stability:
- Store QUANTI-Blue™ pouches at 2-8°C for 12 months.
  Important: The correct storage temperature for this product is 2-8°C (some pouches may be mislabeled).
- Reconstituted QUANTI-Blue™ medium is stable 2 weeks at 2-8°C and 2 months at -20°C. Keep reconstituted QUANTI-Blue™ away from light.

DESCRIPTION
QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ medium changes to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters that are exploited by the use of QUANTI-Blue™.

• Requires small samples of cell supernatants - Samples of 10 µl are sufficient.
• No need to process samples - Preparation of cell lysates or heating of samples are not required.
• Determine secreted AP activity without disturbing cells - The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation.
• Assay can be completed in 30 min - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™.
• Wide dynamic range allows to detect low and high levels of AP content without disturbing cells
• Highly sensitive for quantitative measurement
Higher saturation threshold than with pNPP resulting in more significant differences between non or low AP expression and high AP expression.
• Extremely simple to use - QUANTI-Blue™ consists of only one medium: 1) resuspend in water, 2) add sample, incubate at 37°C and 3) assess AP activity with the naked eye or by reading the optical density (OD) at 625-655 nm.

METHODS
Preparation of QUANTI-Blue™
- Pour the contents of one pouch of QUANTI-Blue™ in a 250 ml sterile glass bottle or flask.
- Add 100 ml of endotoxin-free water.
- Swirl gently.
- Warm QUANTI-Blue™ to 37°C for 30 min.
- Use reconstituted QUANTI-Blue™ immediately or store at 2-8°C.

Notes:
- QUANTI-Blue™ may require overnight incubation at 2-8°C to ensure complete dissolution of the powder.
- Optional: To guarantee sterility, QUANTI-Blue™ can be filtered on a 0.2 µm membrane once complete dissolution is achieved. However, this step is not necessary as your cells will not be in contact with QUANTI-Blue™.

Detection of SEAP activity from cell culture supernatants
The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells. Some fetal bovine serum (FBS) may contain alkaline phosphatase that can interfere with SEAP quantification. We recommend to test the culture medium supplemented with FBS as a negative control to evaluate the presence of alkaline phosphatase in the serum.

- Aliquot 200 µl QUANTI-Blue™ per well.
  Note: Warm QUANTI-Blue™ to 37°C before use.
- Add 20 µl supernatant of SEAP-expressing cells or cell culture medium as a negative control.
  Note: If the negative control turns purple/blue, it means your FBS contains alkaline phosphatase. We recommend to heat the FBS used in your cell culture medium at 56°C for 30 minutes to inactivate the alkaline phosphatase activity.
- Incubate at 37°C.
- After 15 min to 24 h incubation, assess SEAP activity with the naked eye or by reading the OD at 620-655 nm with a microplate reader.

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<td>Recombinant SEAP Protein</td>
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InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com